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Manuscript Draft

Manuscript Number: FOODCONT-D-15-01532R1

Title: Characterization and suitability of polyphenols-based formulas to replace sulfur dioxide for storage of sparkling white wine

Article Type: Research Paper

Keywords: antioxidant formula; phenols; sparkling white wine; storage; sulfur dioxide

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Abstract: The sparkling wine protection against air is of interest for maintaining its sensorial profile and it is achieved through the use of antioxidants while disgorging. Sulfur dioxide (SO₂) is commonly added, but its amount should be limited due to human health problems. The suitability of three polyphenols-based commercial formulas containing plant gallic and ellagic acids extracted from grape (*Vitis vinifera* L.) (AO1), plant ellagic acid and gum arabic (AO2), and plant gallic, ellagic acids and *Saccharomyces cerevisiae* cell-wall fractions (AO3) was evaluated after 7 months storage (at 15°C and 25°C) of disgorged sparkling white wine. The phenolic composition of these formulas was investigated through spectrophotometric measurements. Moreover, the phenols were characterized and quantified by HPLC-MS analyses. The sotalon concentration and the absorbance values at 420 nm were determined in wines. The HPLC-MS analysis showed that the formula AO1 mainly contained gallotannins, ellagic tannins and flavan-3-ols, while AO2 had high levels of flavan-3-ols and gallotannins. Flavan-3-ols were the only phenols found in AO3. The addition of these formulas increased the yellow hue. Sotalon was higher than the perception threshold in the samples with AO2 and at trace amount in the samples with both AO1 and AO3 only stored at 25°C. The tested antioxidant formulas seemed to be less effective of SO₂ for the storage of sparkling white wine. However, the investigation of phenolics in antioxidant formulas could be helpful for the proper choice of a potential substitute of SO₂ due to increase interest in sulfur-free wine production.

Subject: submission of the manuscript: “Characterization and suitability of polyphenols-based formulas to replace sulfur dioxide for storage of sparkling white wine”

Dear Editor,

I have the pleasure to send you the revised paper in the subject for review. The authors are:

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The manuscript was modified following the reviewers' comments and suggestions. The identification of the single phenols in the antioxidant formulas was highlighted and the style was also changed.

Moreover, we would apologize for the several mistakes in the tables numbering in the text.

Faithfully,

Dr. Daniela Fracassetti

Reviewers' comments:

Reviewer #1: Looking how much effort and detail were put into the characterization of the commercial antioxidant products, I think the focus of the article should be shifted a bit towards that aspect. Meanwhile, the evaluation of the treated wines is an application of these products, and the authors tried to relate the findings in the wines back to the content of the additives. I think this subtle shift in focus could be reflected in the title, abstract and introduction.

One issue that I have highlighted in the reviewed version of the text is the use of TP and TF for total phenolics as measured by the FC assay and total flavonoids (in fact phenolics) measured by absorbance. This issue has to be addressed by the authors, since it is misleading.

The Folin-Ciocalteu reagent allows the determination of the total phenols index. The measurement of total flavonoids by the method described by Di Stefano, Cravero, & Gentilini (1989) allows also the estimation of the non-flavonoids content. In this method, the direct reading at 280 nm (total flavonoids, TF) is required and it can be subtracted by the absorbance value found for the proanthocyanidins corrected at 280 nm.

The text was modified in order to clarify the two methods used obtaining a deeper indexes of the phenolic contents on the antioxidant formulas investigated.

There are some comments on the style of the paper I would like to make: even though the language is good, the use of some English terms and phrases in the scientific context is a bit forced. The most often encountered is "added with" - which should be replaced, depending on the context, with "containing", "addition" and such (I have made some of these suggestions in the text).

Some of the phrases are long. The use of the semicolon (;) is extensive in some parts of the text - I recommend to replace it with a full stop, since this is a scientific text.

The table numbers don't always correspond to the table that presents the information discussed.

As the reviewer suggested, the manuscript was entirely revised emphasizing the characterization of the polyphenols-based formulas in the title, abstract and introduction. The style of the paper was also modified.

Reviewer #2: Comments:

This manuscript titled "Suitability of polyphenols-based formulas to replace sulfur dioxide for storage of sparkling white wine" studies the use of the three different antioxidant formulas added to an Italian sparkling white wine (Champenoise method) while disgorging as potential substitutes of SO₂. This manuscript displays a new and really interesting information. Furthermore, the manuscript is well built and written.

However I have some comments/doubts so authors can see below a point by point:

Line: 47 Please correct "(Guichard, Pham, &Etievant, 1999)" to "(Guichard, Pham, &Etievant, 1993)"

The reference was changes, as the review suggested

Line: 60 provide a reference to support this statement (2-ketobutyric acid formation)

The reference was added (Pons, Lavigne, Landais, Darriet, &Dubourdieu(2010) Journal of Agricultural and Food Chemistry, 58, 7273-7279).

Line: 220 Reactivity to sulfur dioxide: provide a reference a support

The reference was added (Di Stefano, &Cravero, 1991).

Lines: 270 Total phenols in sparkling wine: provide a reference a support. Why they were not determined the total polyphenols to FolinCiocalteu?

The polyphenols index was determined in wine in accordance to Di Stefano, Cravero, &Gentilini, 1989. This method provides a suitable determination of the phenols concentration in wine.

Lines: 292, 294, 300, 310, 320 review in the text the references of the tables (table 2 to table 3 or 4).

The number of tables was modified in the text.

Line: 326 Please correct "(1.51 g/100 g powder)" to "(1.50 g/100 g powder)".

The quantification reported into the manuscript was corrected.

Lines: 329, 334 Please correct "table 3" to "table 4"

Lines: 330, 335, 375, 393 Please correct "table 1" to "table 2"

Lines: 332, 333, 338, 340, 341 Please correct "table 4" to "table 5"

The number of tables was modified in the text.

Lines: 401- 403 provide a reference a support

The statements reported in these lines are not supported by the literature. As we mentioned "The rationale behind the increased GSH content is not clear." The following statement is an "hypothesis" as we clarified since we found the "GRP decreased over the storage and lower concentration of this compound was found at 25°C in comparison to 15°C".

Line: 406 concentration of GSH (5.8 g/100 g)? or 5.8 mg/100 g

The GSH concentration as 5.8 g/100 g is correct.

Line: 546 Please correct "table 1" to "table 4"

The number of table was modified in the figure caption.

Lines: 548, 549 add in the Table 1 the standard deviation

As the reviewer suggested, the standard deviation was added in table 1.

Line: 551 Table 2: Please correct "SO2" to "SO₂"

This correction was carried out in table 2.

Line: 561 Table 5: Please correct "Glutatione" to "Glutathion". Concentration of GSH (5.8 g/100 g)?or 5.8 mg/100 g

"Glutathione" was corrected in the table.

Line: 567 Table 6 Please correct "Glutatione" to "Glutathion"

"Glutathione" was corrected in the tablefootnote.

1 | **Characterization and Suitability of polyphenols-based formulas to replace sulfur dioxide for**
2 **storage of sparkling white wine**

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11 Short title: Polyphenols formulas as sulfur dioxide alternative

12

13 Abstract

14 The sparkling wine protection against air is of interest for maintaining its sensorial profile and it is
15 achieved through the use of antioxidants while disgorging. Sulfur dioxide (SO₂) is commonly
16 added, but its amount should be limited due to human health problems. The suitability of three
17 polyphenols-based commercial formulas containing plant gallic and ellagic acids extracted from
18 grape (*Vitis vinifera* L.) (AO1), plant ellagic acid and gum arabic (AO2), and plant gallic, ellagic
19 acids and *Saccharomyces cerevisiae* cell-wall fractions (AO3) was evaluated after 7 months storage
20 (at 15°C and 25°C) of disgorged sparkling white wine. The phenolic composition of these formulas
21 was investigated through spectrophotometric measurements. ~~and~~ Moreover, the phenols were
22 characterized and quantified by HPLC-MS analyses. The sotolon concentration and the absorbance
23 values at 420 nm were determined in wines. The HPLC-MS analysis showed that ~~T~~he formula
24 AO1 mainly contained gallotannins, ellagic tannins and flavan-3-ols, while AO2 had high levels of
25 flavan-3-ols and gallotannins. Flavan-3-ols were the only phenols found in AO3. The addition of
26 these formulas increased the yellow hue. Sotolon was higher than the perception threshold in the
27 samples ~~added~~ with AO2 and at trace amount in the samples with both AO1 and AO3 only stored at
28 25°C. The tested antioxidant formulas seemed to be less effective of SO₂ for the storage of
29 sparkling white wine. However, the investigation of phenolics in antioxidant formulas could be
30 helpful for the proper choice of a potential substitute of SO₂ due to increase interest in sulfur-free
31 wine production.

32 **Keywords:** antioxidant formulas, phenols, sparkling white wine, storage, sulfur dioxide.

33

34 1. Introduction

35 Disgorging and corking are critical steps in sparkling wine production because the wine can be
36 easily exposed to the air which leads to oxygen dissolution. Oxygen can worsen the sensorial
37 properties of sparkling wine and shorten the shelf life because it can degrade some aromatic esters
38 and terpenes (Roussis, Lambropoulos, & Tzimas, 2007) and it can speed up the formation of
39 compounds with oxidized off-odor such as sotolon (4,5-dimethyl-3-hydroxy-2,5-dihydrofuran-2-
40 one) (Lavigne, Pons, Darriet, & Dubourdieu, 2008).

41 Sotolon odor is perceived as a defect in young dry white wine since it decreases the intensity of the
42 fruity and flowery notes as well as the expected freshness character (Silva Ferreira, Barbe, &
43 Bertrand, 2003). Sotolon can arise from the aldol condensation of 2-ketobutyric acid and ethanal
44 (Kobayashi 1989, König et al. 1999; Cutzach, Chatonnet, & Dubourdieu, 1999), as well as from the
45 Maillard reaction (Pons, Lavigne, Landais, Darriet, & Dubourdieu, 2010) and the oxidative
46 degradation of ascorbic acid in a hydro-alcoholic solution (König et al. 1999). These pathways are
47 quantitatively favored as the concentrations of oxygen and reducing sugars increase (Cutzach et al.
48 1999; Camara, Marques, Alves, & Silva Ferreira, 2004; Lavigne et al. 2008). Its perception
49 threshold in white wine was reported to be 7-8 µg/l (Guichard, Pham, & Etievant, 1993³⁹) and
50 sotolon might be adopted as a chemical marker of oxidative aging.

51 In order to avoid oxidation of aromatic compounds and the formation of oxidized off-flavors, sulfur
52 dioxide (SO₂) is commonly added to sparkling white wine while disgorging since this compound is
53 rapidly oxidized to sulfate by an oxidation/reduction cycle of hydroxycinnamoyl tartaric acids
54 (Danilewicz, 2003). As a consequence, the dissolved oxygen can be consumed quicker in presence
55 of this antioxidant (Danilewicz, 2011). Though SO₂ is useful to limit the oxidative damage of white
56 wine, its amount should be limited because of the detrimental effect on human health and the
57 intolerance shown by a number of wine consumers, mainly asthmatics (Lester, 1995; Vally &
58 Thompson, 2001; Pozo-Bayon, Monagas, Bartolomé, & Moreno-Arribas, 2012). Therefore, other

antioxidant compounds safer to human health should be considered and tested in wine making. Ascorbic acid could be effective to this aim (Marks & Morris, 1993) due to its low redox potential (Danilewicz 2003), but its oxidation gives rise to both hydrogen peroxide (Riberau-Gayon, Glories, Maujean, & Dubourdieu, 2006) and 2-ketobutyric acid [\(Pons et al., 2010\)](#). Glutathione (GSH) showed to be effective in decreasing sotolon formation in the oxidative aging of barreled white wine (Lavigne & Dubourdieu 2004). Nevertheless, high concentrations of GSH might need to be effective, but its average amounts in wine hardly exceed few milligrams per liter (Cassol & Adams, 1995; du Toit, Lisjak, Stander, & Prevoo, 2007; Fracassetti & Tirelli 2015). Oxygen in wine can also be consumed by polyphenols due to their low redox potential. Polyphenols containing trihydroxyphenyl groups (i.e. galloylated phenols) have a lower redox potential than polyphenols containing dihydroxyphenyl groups and they can completely deplete oxygen from wine (Danilewicz, 2011; Danilewicz, 2012). White wine usually contains negligible amounts of trihydroxyl substituted phenyl compounds and the addition of mixtures containing phenols into the wine might limit the oxidative reactions in sparkling white wine during shelf life. Recently, the use of plant phenolics extract was shown to be effective as an alternative to SO₂ in white wine aged in barrels (González-Rompinelli et al., 2013). The addition of gallotannins showed to play a positive role in the maintenance of esters in white wine after 1 year storage (Sonni, Chinnici, Natali, & Riponi, 2011). However, it is known that astringency and bitterness are affected to high concentration of tannins, but their perception is strictly dependent to the phenols concentration (Robichaud & Noble, 1990). The effectiveness of polyphenols-based preparation needs to be elucidate since no data are available related to their phenolic [content and the nature of the single phenols. The knowledge of the phenols composition can be helpful for better comprehend the effect of these antioxidant preparation in wine. ~~composition and~~ The investigation of](#) the consequences on oxidative damage of sparkling white wine in comparison to SO₂ [is also required](#).

On this purpose, this study was aimed to investigate the addition of three different antioxidant formulas added to an Italian sparkling white wine (*Champenoise* method) while disgorging as potential substitutes of SO₂. The phenolic composition of these antioxidant formulas was attentively characterized by spectrophotometric and HPLC-MS analysis. The latter allowed the identification and quantification of the single phenolic compounds. ~~and~~ The levels of sotolon and GSH, and the changes of color were also evaluated. To the best of our knowledge, the phenolic composition of industrially-produced antioxidant formulas for oenological purpose has never been investigated as well as their effect throughout sparkling wine storage.

2. Material and Methods

2.1 Chemicals

All the chemicals were of analytical grade. 3-Mercaptopropionic acid (3MPA) and p-benzoquinone (pBQ) were purchased from Fluka (Switzerland). Glutathione (GSH), cysteine (Cys), sotolon, ascorbic acid (AA), dehydroascorbic acid (DHA), 1,2-phenylenediamine dihydrochloride (OPDA), dichloromethane (DCM), FeSO₄·7 H₂O, sodium chloride (NaCl), anhydrous sodium sulphate and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Polyvinylpyrrolidone (PVPP) was purchased from Dal Cin (Sesto San Giovanni, Milan, Italy). Citric acid was purchased from J. T. Baker (Phillipsburg, NJ, US); HPLC grade methanol was from Panreac (Barcelona, Spain), and HPLC grade water was obtained by a Milli-Q system (Millipore Filter Corp., Bedford, MA, USA). The synthetic wine solution contained 5 g/l tartaric acid in 12% ethanol/water solution (v/v), adjusted to pH 3.5 with 12 M sodium hydroxide (Sigma-Aldrich). Three commercial powders containing phenolics as antioxidant purpose for the winemaking use were purchased on the market. These formulas were labeled as mixtures of plant gallic and ellagic acids extracted from grape (*Vitis vinifera* L.) (sample coded as AO1), plant ellagic acid and gum

108 arabic (sample coded as AO2), and plant gallic, ellagic acids and *Saccharomyces cerevisiae* cell-
109 wall fractions (samples coded as AO3).

110

111 2.2 Sparkling wine samples

112 The sparkling white wine was industrial-scale produced by a cellar located in the Franciacorta area
113 (Lombardy, Italy) in the 2010 vintage from Chardonnay grape. The rational wine making
114 procedures usually adopted in the winery for the manufacture of *Champenoise* sparkling wine were
115 followed and no addition of SO₂ was carried out. Base wine (10 hl) was bottled, the second
116 fermentation was performed and the sparkling wine was maintained 12 months on the yeast lees
117 before the disgorging.

118

119 2.3 Experimental design

120 Sulfur dioxide (50 mg/l) and the three antioxidant formulas (20 mg/l and 40 mg/l) were separately
121 added to bottled sparkling white wine samples after *à la glace* disgorging. The bottles were
122 manually filled with 10 ml of the same sparkling white wine containing the antioxidant in order to
123 reach the final volume of 750 ml and they were closed with crown cap. Control samples were
124 disgorged, filled with sparkling white wine antioxidant-free and capped. The chemical parameters
125 of both base wines (control and test) are reported in Table 1 and only negligible differences were
126 found. All the bottles were stored for 7 months in two different rooms at 15°C and 25°C in the dark.
127 For each treatment and temperature investigated, the content of GSH, sotolon, AA and DHA, and
128 the absorbance values at 420 nm were evaluated. Each trial was performed in duplicate.

129

130 2.4 Determination of sotolon

131 Sotolon was measured in both sparkling wines and antioxidant formulas. The wine samples
132 preparation was carried out as described by Gabrielli, Fracassetti and Tirelli (2014). Briefly, 3 g of

NaCl were dissolved in 30 ml wine in a 100 ml bottle then 40 ml of dichloromethane (DCM) were added. The bottle was hermetically closed and shaken for 10 min with a wrist action stirrer (Griffin Flask Shaker). The mixture was centrifuged 5 min at $5000 \times g$ and the DCM was separated by a separatory funnel and recovered. This solvent extraction procedure was carried out for 3 times. The organic solvent fractions were jointly collected and ~~added with~~ 2 g of anhydrous sodium sulfate were added. The DCM was evaporated under-vacuum and the dry material was dissolved with 2 ml of methanol 5% which was purified by a PVPP 50 mg SPE cartridge and recovering the eluted solution ~~was recovered~~. For the antioxidant formulas, 200 mg of powder were dissolved in 50 ml of the synthetic wine solution. The liquid/liquid extraction of sotolon was carried out as reported as above for the sparkling wine samples. Each wine sample and formula was analysed in triplicate.

2.5 Determination of glutathione and free and adsorbed cysteine

Glutathione was evaluated in both sparkling wines and commercial formulas. For the sparkling wine samples, its content was determined as described by Fracassetti and Tirelli (2015). Briefly, the sparkling wine (2 ml) treated with PVPP and centrifuged was derivatised with pBQ followed by the addition of 3MPA. The reaction mix was filtered through 0.22 μm pore size PTFE membrane (Millipore, Billerica, MA, USA) and the HPLC analysis was performed. Glutathione in antioxidant formulas was measured as described by Tirelli, Fracassetti and De Noni (2010). Briefly, the powders were suspended in citrate buffer 75 mmol/l at pH 5 for GSH and Cys determination and in citrate buffer 75 mmol/l at pH 5 where Cys (5 mg/l) for adsorbed Cys, derivatised with pBQ and ~~added with~~ 3MPA was added. The reaction mix was filtered through a 0.22 μm pore size PTFE membrane (Millipore) and submitted to the HPLC separation. The GSH and Cys content in the antioxidant formulas was directly quantified by the HPLC analysis, while the Cys absorbed by the powders was determined by difference with the response (peak area) obtained injecting Cys 5 mg/l

158 dissolved in citrate buffer 75 mmol/l at pH 5. Each wine sample and powder was analysed in
159 triplicate.

160

161 2.6 Determination of ascorbic acid and dehydroascorbic acid

162 Quantification of AA and DHA was carried out as previously described by Zapata and Dufour
163 (1992) with some modifications. Thirty milliliters of wine and 100 mg of the antioxidant formula
164 dissolved in 100 ml of synthetic wine solution ~~added with~~containing EDTA (0.03%). The samples
165 were filtered through a 0.45 µm PVDF filter and purified on a C18 Sep-Pak cartridge (Waters, Mil-
166 ford, MA, US). The HPLC analysis was carried out after derivatisation of DHA into the fluorophore
167 3-(1,2-dihydroxyethyl) furol [3,4-b]quinoxaline-1-one (DFQ), with OPDA. Standard solutions of
168 both AA and DHA ranged from 2 mg/l to 50 mg/l were prepared in synthetic wine solution.
169 Reversed phase HPLC separation was performed with a Waters Alliance 2695 (Milford, MA, US)
170 equipped with a photodiode array detector Waters 2996 and a C18 column (Nova-Pak 150 x 3.9
171 mm, 4 µm, Waters). The chromatographic separation was carried out with an isocratic elution
172 running acetate buffer 50 mmol/l, at pH 4.5/~~added with 5%~~ methanol 95/5 (v/v) for 15 min
173 followed by column washing (100% methanol for 2 min) and column conditioning (4 min). The
174 flow rate was 0.9 ml/min. Column temperature was 25°C and the injection volume was 20 µl.
175 Chromatographic data were registered from 230 nm to 500 nm and processed at 261 nm and 348
176 nm respectively for AA and DHA by Empower 2 software (Waters). Each formula was analysed in
177 triplicate.

178

179 2.7 Antioxidant capacity assays

180 The antioxidant capacity of the antioxidant formulas was carried out both DPPH and ABTS assays.
181 The free radical scavenging activity determined with DPPH assay followed the method of Brand-
182 Williams, Cuvelier and Berset (1995) with some modifications (Espín, Soler-Rivas, Wichers, &

183 García-Viguera, 2000; Llorach, Tomás-Barberán, & Ferreres, 2004). The DPPH solution was
184 diluted with methanol to obtain 1.00 ± 0.03 absorbance units at 515 nm. In a 96-wells micro plate
185 (Nunc, Roskilde, Denmark), 250 μ l of DPPH solution were placed in each well and 2 μ l sample
186 were added. The sample was dissolved in 70% methanol (20 g/l) and, after centrifugation, it was
187 serially diluted. The ABTS method was performed as reported by Mena et al. (2011). The ABTS
188 solution was diluted with water to obtain 1.00 ± 0.03 absorbance units at 414 nm. In a 96-wells
189 micro plate (Nunc, Roskilde), 250 μ L of ABTS solution were put in each well and 2 μ l sample were
190 added. The sample was dissolved in water (20 g/l) and, after centrifugation, it was serially diluted.
191 For both assays, the reaction kinetic was monitored for 50 min at 25°C by micro plate reader
192 (Infinite® M200, Tecan, Grödig, Austria). A calibration curve was made by adding increasing
193 concentration of Trolox ranged from 50 to 1000 μ mol/L. Each concentration was assayed in
194 quadruplicate, as well each sample. Results were expressed as mol Trolox per 100 g of powder.

195

196 2.8 Determination of phenolic compounds in the antioxidant formulas

197 2.8.1 Spectrophotometric analysis

198 The total phenols (TP) level of the antioxidant formulas was estimated colorimetrically by Folin-
199 Ciocalteau method (Scalbert, Monties, & Janin, 1989). The formulas (1 g/l) were dissolved in
200 methanol/water 50/50 (v/v) and diluted 2.5, 5 and 10 times in the same solvent. The Folin-
201 Ciocalteau reagent was diluted 10 times in water (v/v) and 2.5 ml was added to 0.5 ml of sample.
202 Two milliliters of 75 g/l sodium carbonate solution were added and the tubes were kept one hour at
203 room temperature in the dark. In the meanwhile, the calibration curve for gallic acid (5-100 mg/l)
204 dissolved in methanol/water 50/50 (v/v) was achieved. The absorbance at 765 nm was measured
205 and the results were expressed as g gallic acid/100 g powder. Each formula was analysed in
206 triplicate.

207 In order to investigate deeply on the phenols in these formulas, The total flavonoids (TF) and non-
208 flavonoids (NF) contents of the antioxidant formulas ~~was~~were also determined in accordance with
209 Di Stefano, Cravero, and Gentilini (1989). The formulas (1g/l) were dissolved in synthetic wine
210 solution, diluted in chloridric-ethanol solution (ethanol/water/chloridric acid 70/30/1 v/v/v) and the
211 absorbance at 280 nm was measured. The TF concentration was expressed as mg gallic acid/g
212 powder obtained through a calibration curve of gallic acid dissolved in the chloridric-ethanol
213 solution (50-200 mg/l). Each formula was analyzed in triplicate. The ~~non-flavonoids (NF)~~
214 concentration was estimated ~~as reported by Di Stefano et al. (1989)~~ by subtracting to the absorbance
215 value from TF the absorbance value found for the proanthocyanidins (see below) corrected at 280
216 nm. It was expressed as g gallic acid/100 g powder.

217

218 2.8.2 Determination of proanthocyanidins

219 Proanthocyanidins were assessed as described by Bate-Smith (1981). The antioxidant formulas (1
220 g/l) were dissolved in the synthetic wine solution. In two separate test tubes (reaction tube and
221 blank tube) 2 ml of sample, 10.5 ml of ethanol and 12.5 ml of hydrochloric acid 37% (v/v)
222 containing 300 mg/l of $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ were added. The reaction tube was placed in a water bath at
223 100 °C for 50 min, while the blank tube was left to stand in the dark in ice. After 50 min, the
224 reaction tubes were cooled in ice for 10 min. The absorbance was measured at 550 nm. The
225 concentration of proanthocyanidins was calculated multiplying the absorbance difference among the
226 reaction tube and the blank tube by the factor 1162.5 and results were expressed as g cyanidin /100
227 g powder (Di Stefano et al., 1989). The determination was carried out in triplicate.

228

229 2.8.3 Reactivity to sulfur dioxide

230 The reactivity to SO_2 of powders was determined by spectrophotometric analysis in order to assess
231 the oxidized phenols which higher concentrations lead to an increase of absorbance in presence of

232 | SO₂ [\(Di Stefano & Cravero, 1991\)](#). The formulas (1 g/l) were dissolved in the synthetic wine
233 solution and the absorbance at 280 nm was measured before and after the addition of SO₂ (0.3%).
234 Water was used as blank. The difference of absorbance values between the readings carried out
235 before and after the addition of SO₂ was expressed as g gallic acid reactive to SO₂ per 100 g of
236 powder through a calibration curve of gallic acid dissolved in the synthetic wine solution (50-500
237 mg/l). The determination was carried out in triplicate.

238 239 2.8.4 Determination of *o*-dihydroxyl and *o*-trihydroxyl phenols

240 The *o*-dihydroxyl and *o*-trihydroxyl phenols were spectrometrically determined, as described by
241 Riberau-Gayon (1968). The method took into account the different absorbance response at 545 nm
242 of *o*-dihydroxyl (pyrocatechol) and *o*-trihydroxyl phenols (pyrogallol) dissolved in reaction buffer
243 (sodium and potassium tartrate 5 g/l, FeSO₄ 1 g/l) after addition of borate buffer (12.37 g/l boric
244 acid, 14.91 g/l potassium chloride, pH 8.1-8.3 adjusted with NaOH 1 N) or acetate buffer (10%
245 ammonium acetate, pH 8.1-8.3 adjusted with ammonium hydroxide 10%). The content of *o*-
246 dihydroxyl and *o*-trihydroxyl phenols was expressed as percentage. The determination was carried
247 out in triplicate.

248 249 2.8.5 LC-MS analysis

250 The phenols characterization was carried out on the three antioxidant formulas added to the
251 sparkling wine. The phenolic compounds were identified and quantified as reported by Fracassetti,
252 Costa, Moulay, & Tomás-Barberán (2013). The extraction of phenolics was performed as follows: 1
253 g of the formulas was added to 25 ml of methanol/water 50/50 acidified with 1% formic acid. The
254 formulas were vortexed for 2 min, sonicated for 15 min (Sonicator Branson 5510, Emerson,
255 Danbury, CT, US) and centrifuged at 5000 x g for 15 min at 4°C (Centrifuge 5804 R, Eppendorf,
256 Hamburg, Germany). The supernatants were recovered, freeze-dried under vacuum, suspended in 2

257 ml of the corresponding extraction solvent, then filtered with a PVDF filter 0.22 μm (Millipore) and
258 injected in LC-MS. The identification and quantification of phenols were performed using an
259 Agilent 1100 Series equipment (Agilent, Santa Clara, CA, USA) equipped with G1312A binary
260 pump, G1313A autosampler, G1315B photodiode array detector, and G1322A degasser controlled
261 by the Agilent software v. A08.03. HPLC was coupled with a detector MSD Trap 1100 Series
262 (Agilent) with an electrospray ionisation system (ESI), with the following conditions: the heated
263 capillary was 350°C and 3–3.5 kV voltage, mass scan (MS) and MS/MS were measured from 100
264 to 1500 m/z . Collision induced fragmentation experiments were performed in the ion trap using
265 helium as the collision gas, and the collision energy was set at 75%. Mass spectrometry data were
266 acquired in the negative ionisation mode. A column Pursuit XRs C18 250x40 mm from Varian
267 (Agilent) was used and a flow rate of 0.8 ml/min. The used solvents were 1% formic acid in water
268 (A) and acetonitrile (B) which was in the following separation gradient: 1% B in A at 0 min, 9% B
269 at 10 min, 35% B at 48 min, and 95% B at 52 min, following by washing and conditioning steps.
270 Data were registered from 250 nm to 700 nm and the phenolic compounds were quantified at 280
271 nm, 360 nm, and 520 nm, depending on the type of phenolic compound. Integrations were
272 performed by Agilent ChemStation for LC 3D, Rev. B.01.03 SR1. MS trap control was carried out
273 Bruker Daltonic version 5.2. Quantification of gallic acid, ellagic acid, myricetin and their
274 derivatives, and ellagitannins was carried out with the calibration curves obtained for gallic acid (1–
275 300 mg/l), ellagic acid (1–300 mg/l), rutin (1–300 mg/l), and vescalagin (0.1–100 mg/l),
276 respectively, at the appropriate wavelengths. All the samples and standards were injected in
277 triplicate.

278

279 2.9 Total phenols in sparkling wine

280 The total phenols concentration in sparkling wine samples was assessed through spectrophotometric
281 analysis recording the absorbance at 280 nm ([Di Stefano et al., 1989](#)). The data were expressed as

282 mg gallic acid/L obtained through a calibration curve of gallic acid dissolved in synthetic wine
283 solution (50-200 mg/l). The analyses were carried out in triplicate.

284

285 2.10 Statistical analyses

286 The one-way ANOVA was performed using STATISTICA 9 software (Statsoft Inc., Tulsa, OK,
287 US). Significant differences were judged to using a 5% significance level ($p < 0.05$). The
288 correlation coefficients between GSH, GRP, sotolon and the absorbance at 420 nm were computed
289 through the Pearson correlation.

290

291 3. Results and Discussion

292 3.1. Characterization of the commercial antioxidant formulas

293 The phenolics of the antioxidant formulas were characterized in order to achieve more detailed
294 composition of them. The TP concentrations determined by the Folin-Ciocalteu reagent
295 corresponded to 58% and 51% for the formulas AO1 and AO2, respectively (Table 2). Lower
296 amount of TP was detected in AO3 (14.2%). The presence of polymeric (as proanthocyanidins) and
297 monomeric phenols was evaluated. Flavan-3-ol polymers were most abundant in AO2 (19.0%) and
298 not detectable in AO3. The formula AO1 showed highest amounts of both TF (39.5%) and non-
299 flavonoids (37.5%).

300 In order to achieve a deeper knowledge of the antioxidant formulas employed for this research, the
301 low-molecular weight phenols were characterized by LC-MS as shown in Table 23 and Figure 1.
302 All the compounds were characterized by their UV spectra and their molecular ion and fragments
303 obtained with an ESI-MS/MS detector (Table 23) and comparison, wherever possible, was carried
304 out with standard compounds. Flavonols, ellagic acid conjugates, ellagitannins and
305 proanthocyanidins were the most represented polyphenols. Quercetin (3,5,7,3',4'-
306 pentahydroxyflavone) (56) and its 3-O-glycoside (53) were detected, the latter in AO3 only. They

showed the characteristic UV spectra of flavonols with a free hydroxyl group at position 3 for quercetin (UV band I maximum at 370 nm), as well as its glycosylated form at position 3 (UV band I maximum at 356 nm) (Table 23). The pseudomolecular ions recorded with the HPLC-ESI MS and the fragments obtained confirmed these structures with the characteristic losses of a glycosyl residue respectively leading to the quercetin aglycone fragment at m/z 301. Kaempferol (3,4',5,7-tetrahydroxyflavone) (57) and its 3-*O*-glycosyl derivative (54) were revealed, the latter only in AO3. This compound showed m/z 755 and it is probably a hexoxyl-rhamnosyl-hexoside derivative of kaempferol. In addition, myricetin (3,5,7,3',4',5'-hexahydroxyflavone) (55) was detected in AO3 only. The isomeric ellagitannin *C*-glucosides vescalagin (2) and castalagin (4) were characterized by both the pseudomolecular ion at m/z 933 and the characteristic fragments that did not include the ellagic acid fragment at m/z 301 as they were *C*-glycosides. These two phenols were confirmed by chromatographic comparisons with their respective standards. Ellagic acid (33) and ellagitannins hexahydroxy-diphenoyl-galloyl-glucose isomers (5, 6) were detected (Table 23) only in AO1 as they showed the characteristic UV spectrum of ellagic acid and ellagitannins. Among the latter two phenols, the main one was free ellagic acid that showed a pseudomolecular ion at m/z 301 and it overlapped chromatographically with an authentic standard of this phenol. Several hydrolysable tannins, mainly gallotannins, were particularly abundant in AO1, some of these compounds were also revealed in AO2, while none of them was found in AO3. Most of the hydrolysable tannins were recognized as galloyl derivatives of quinic acid through the comparison of the molecular weight with both parent and daughter ions and UV spectra (Clifford, Stoupi, & Kuhnert, 2007). Gallic acid (1) was also detected and it was confirmed by the chromatographic analysis of the standard compound. Flavan-3-ols were also present in these antioxidant formulas with some differences among them (Table 23). Gallocatechin (3) a dimer of catechin (11) and gallocatechin gallate (17) were revealed only in AO3. A dimer of catechin gallate (35) was found in AO1, AO2

331 and AO3. A dimer of gallocatechin (8) was detected in AO2 and AO3. The three antioxidant
332 formulas contained a dimer of catechin (10) and a dimer of gallocatechin gallate (14).
333 The antioxidant formula AO1 contained the highest level of low molecular weight phenols (15.8
334 g/100 g powder) and the gallotannins were the most abundant compounds (70%) (Table 34). Ellagic
335 acid and ellagitannins were detected only in such formula (1.540 g/100 g powder). Gallic acid (1.15
336 g/100 g powder) and proanthocyanidins (2.11 g/100 g powder) were also found as well as flavonols
337 (0.04 g/100 g powder), namely quercetin and kaempferol. The data obtained by LC-MS confirm the
338 high level of hydrolyzable tannins in AO1 mainly represented by trihydroxyl phenols (Table 34) as
339 found also spectrophotometrically (Table 42). The high concentration of phenolic compounds in
340 AO1 could ease an effective consumption of oxygen (Danilewicz, 2011). The antioxidant activity
341 values of the formulas tested (Table 45) seemed to confirm this hypothesis and were proportional
342 with the TP levels. The ratio value DPPH/TP (Table 45) showed a poor antioxidant ability of AO2
343 polyphenols, in spite of the gallotannins presence (Table 34), when it was compared to same value
344 of AO3 formula which did not contain gallotannins (Table 14). This could indicate the presence of
345 oxidized phenols in AO2 as also suggested by the spectrophotometric analysis showing a higher
346 level of phenols reactive to SO₂ in comparison to AO1 and AO3 (Table 2). The presence of *o*-
347 quinones could be indicated by the presence of absorbed Cys revealed in AO2 (Table 45) since
348 these compounds have a strong reactivity with the thiols (Riberau-Gayon et al. 2006). Cys was
349 absorbed even by AO3 (Table 45) and the ability of binding the Cys could partly explain the low
350 ratio values DPPH index/TP and ABTS/TP found for both AO2 and AO3 (Table 54). The levels of
351 GSH and AA were evaluated in order to assess the presence of non-phenolic antioxidants. No
352 antioxidant formula contained AA, whereas GSH was detected only in AO3 (5.8 g/100 g powder).
353 This is in accordance with the presence of yeast cell-wall fractions (Tirelli et al. 2010) as declared
354 by the supplier.

355

3.2. Influence of antioxidant formulas on sparkling white wines

The addition of antioxidant formulas potentially replacing SO₂ was evaluated in sparkling white wine. The use of SO₂ should be minimized owing to its problems for human health (Pozo-Bayón et al., 2012). This compound should be replaced in wine with suitable antioxidant mixtures. The proper amount of the polyphenols-based antioxidant formulas in sparkling white wine was chosen taking into account both technological and sensory factors since AO3 can be responsible for wine haze due to the yeast cell-wall fractions it contained. Antioxidant formulas AO1 and AO2 were mainly constituted with polyphenols which could confer astringency if added in high concentrations (Robichaud & Noble, 1990). Moreover, tannins could react with the wine proteins which lead to haziness and worsen the foaming properties (Coelho, Rocha, & Coimbra, 2011; Martínez-Lapuente, Guadalupe, Ayestarán, & Pérez-Magariño, 2015). Therefore, additions up to 20 mg/l and 40 mg/l of each tested antioxidant formula were carried out~~the sparkling white wines were added with 20 mg/l and 40 mg/l of each antioxidant formula tested~~, as also suggested by the supplier. These additions did not affect the wine astringency since the highest concentration of phenols added was about 23.2 mg/l which was lower than the amount of tannin causing its perception (Robichaud & Noble, 1990; Bertand et al., 2000). The total phenols content ranged from 118.5 mg/l to 147.4 mg/l in wine samples. ~~s~~Significant differences were found due to the addition of both AO1 and AO2 in comparison to control wine samples and ~~added with those samples where~~ SO₂ and AO3 were added (Table 6). This could be due to the own high concentration of total phenols of these formulas (Table 2). The oxidation of phenols to quinones due to air entrance in the bottle could be expected especially in the sparkling wine samples ~~added with~~ containing the formulas ~~containing which~~ showed lower concentration of *o*-trihydroxyl phenols (Danilewicz, 2011).

The addition of SO₂ was the most effective in protecting wine against the oxidation since sotolon was not found and the lowest absorbance values at 420 nm were observed (Table ~~5~~6). Higher absorbance values were revealed in the sparkling wine samples ~~added with~~where the three

antioxidant formulas ~~were added~~, particularly those supplemented with AO2. Sotolon in concentration close to (6.41 µg/l) or higher than (13.37 µg/l) the perception threshold was detected in the wine samples ~~added-with~~where 40 mg/l of AO2 ~~were added~~ and ~~they were~~ stored at 15°C and 25°C, respectively. This finding was not expected since the sparkling white wine samples containing lower amount of polyphenols-based formulas were supposed to consume oxygen at lower rate leading to a lower sotolon concentration. However, AO2 showed the highest level of dihydroxyl phenols (Table ~~24~~) which are responsible for a lower rate of oxygen consumption in comparison to trihydroxyl phenols (Danilewicz, 2011). This suggests that oxygen could participate to other oxidative phenomena. Trace amount of sotolon was detected in the wine samples ~~added with~~containing 20 mg/l of AO2 as well as in the wine samples ~~containing-supplemented with~~ AO1 and AO3 only stored at 25°C. This finding was in accordance to the research carried out by Cutzach, Chatonnet, and Dubourdieu (2000) who reported that high storage temperature (up to 33°C) can promote the formation of sotolon in *Vins doux Naturels*. High storage temperatures can also promote the Maillard reaction which is included among the pathways affecting the sotolon formation (Hoffman, & Schieberle, 1996; Pons et al. 2010). The phenols content did not seem to affect since trace level of sotolon was found in wine samples ~~added-with~~where both AO1 and AO3 ~~were added~~. However, qualitative difference in the phenolics could favor the sotolon formation and further investigations could elucidate the compound(s) majorly affecting the sotolon increase during the storage. The storage temperature seems to play a strong role since significant differences were found in the absorbance values ($p=0.0080$), GSH ($p=0.0002$) and GRP ($p=0.0003$). It also appeared that oxidative phenomena took place in the wine samples ~~added-with~~treated with the antioxidant formulas which seemed to have a negative impact on wine in comparison to SO₂, in terms of off-flavour formation. In fact, minor differences in the absorbance values at 420 nm were noticed in the control wine sample and in the wine samples ~~added-with~~containing AO3 whose phenols concentration was about 4 folds lower than that of AO1 and AO2 (Table ~~12~~).

Besides the formation of brown polymers, a decreased content of GSH was also expected (Salgues, Cheynier, Gunata, & Wylde, 1986). Slight differences were found in the GSH content among the sparkling white wine samples (Table 56). The addition of both SO₂ and the antioxidant formulas did not affect the GSH content in wine. The GSH concentration in wine samples showed an unexpected trend since higher levels were detected in the samples stored at 25°C. Moreover, the GSH content was higher in the samples where higher amounts of antioxidant formulas were added, including those supplemented with AO1 and AO2 which did not contain GSH, in comparison to the antioxidants-free wine samples. The rationale behind the increased GSH content is not clear. As hypothesis, GlutathioneGSH could arise from the glutathionyl-phenols adducts since GRP decreased over the storage and lower concentration of this compound was found at 25°C in comparison to 15°C (Table 56). A positive correlation was found between increased GSH content and decreased GRP content for the different temperatures of storage ($p=0.46$ at 15°C; $p=0.40$ at 25°C). The antioxidant formula AO3 containing GSH (5.8 g/100 g) did not lead to a higher concentration of GSH in in the wine samples where it was added in comparison to the samples supplemented with AO1 and AO2.

4. Conclusions

Our results highlight that the knowledge of the phenolic composition of antioxidant formulas can be helpful for the choice of an appropriate antioxidant mixture in sparkling white wine production. However, the tested polyphenols-based antioxidants were unsuitable to avoid the use of SO₂ as antioxidant in sparkling wine. theyThese formulas seemed to have a detrimental role into the oxidative decay of sparkling white wine whose shelf-life was shorten if compared to sparkling white wine without antioxidant. Our data are not enough to support a correlation between the phenols amount and the oxidative damage though the appearance of the atypical aging was detected

431 | into the wine ~~added with~~containing the formulas tested. Further investigations will need to find an
432 effective antioxidant formula substituting SO₂ while disgorging the sparkling wine allowing the
433 production of sulfur-free wine which has been assuming increasing interest.

434

435 **Acknowledgments**

436 We would like to thank Mr. Francesco Iacono and “Tenuta Fratelli Muratori” winery for providing
437 sparkling white wine samples and the antioxidant formulas.

438 This study was co-financed by the post-doctoral fellow “Dote Ricerca”: FSE, Regione Lombardia.

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559 Figure' captions

560 **Figure 1:** HPLC analyses of phenolic compounds from AO1 at 280 nm (A) and AO3 at 360 nm (B)

561 and at 280 nm (C). For compounds characterization see Table [34](#).

562

563

Parameter	Wine control	Wine test
Ethanol (%)	12.4±0.6	12.3±0.4
Sugar (g/l)	< 2	< 2
pH	3.3±0.1	3.2±0.1
Total acidity (g tartaric acid/l)	6.6±0.3	7.1±0.5
Volatile acidity (g acetic acid/l)	0.43±0.04	0.45±0.02
Free sulfur dioxide (mg/l)	< 5	< 5
Total sulfur dioxide (mg/l)	30±4	20±3

564 Table 1: Chemical composition of the base wines produced in triplicate fermentation.

565

566

Formula	Total phenols index	Total flavonoids	Non-flavonoids	Phenols reactive to SO ₂	Proanthocyanidins	O-dihydroxyl phenols	O-trihydroxyl phenols
	<i>g gallic acid/100 g powder</i>	<i>g gallic acid/100 g powder</i>	<i>g gallic acid/100 g powder</i>	<i>g gallic acid/100 g powder</i>	<i>g cyanidin/100 g powder</i>	%	%
AO1	57.8±3.2	39.48±0.11	37.54±0.11	2.53±0.31	7.84±0.01	9	91
AO2	50.9±9.5	23.10±0.60	17.69±0.59	3.59±0.55	19.00±3.97	51.2	48.8
AO3	14.2±3.3	4.86±0.08	4.86±0.08	2.29±0.31	n.d.	0	100

567 Table 2: Content of the phenolic fractions spectrophotometrically determined in the antioxidant
568 formulas. Data are reported as mean values (n=3) ±standard deviation; n.d.: not detected.

569

Number	Compound	Retention Time (min)	[M-H] ⁺	λ max (nm)	MS fragments
<i>Flavonols</i>					
53	Quercetin 3- <i>O</i> -glucoside	32.2	463	256, 356	301 , 151
54	Kaempferol 3- <i>O</i> -hexosyl-rhamnosyl-hexoside	32.4	755	264, 350	284
55	Myricetin	38.1	317	256, 374	179 , 151
56	Quercetin	45.5	301	256, 370	301, 179 , 151
57	Kaempferol	52.4	285	254, 370	285 , 151
<i>Ellagitannins and ellagic acid</i>					
2	Vescalagin	11.6	933	242	915, 631
4	Castalagin	14	933	242	915, 631
5	Hexahydroxy-diphenoyl-galloyl-glucose	14.5	633	254, 376	301
6	Hexahydroxy-diphenoyl-galloyl-glucose	16.6	633	254, 376	301
33	Ellagic acid	31.5	301	254, 374	301
<i>Gallotannins</i>					
7	Digalloyl quinic acid	17.8	495	236, 274	343 , 269, 169
9	Digalloyl quinic dimer	18.8	991	236, 276	495 , 343, 169, 125
12	Trigalloyl quinic acid	23.1	647	238, 276	495 , 343, 169
13	Trigalloyl quinic acid	23.9	647	238, 276	495 , 343, 169
15	Trigalloyl quinic acid	24.6	647	238, 276	495 , 343, 169
16	Trigalloyl quinic acid	25.2	647	238, 276	495 , 343, 169
18	Digalloyl quinic acid	25.9	495	236, 274	343 , 269, 169
19	Tetragalloyl quinic acid	27.1	799	236, 274	647 , 495
20	Tetragalloyl quinic acid	27.8	799	236, 274	647 , 495
21	Trigalloyl quinic acid	27.9	647	238, 276	495 , 343, 169
22	Tetragalloyl quinic acid	28.1	799	236, 274	647 , 495
23	Tetragalloyl quinic acid	28.5	799	236, 274	647 , 495
24	Trigalloyl quinic acid	28.6	647	238, 276	495 , 343, 169
25	Tetragalloyl quinic acid	29.0	799	236, 274	647 , 495
26	Tetragalloyl quinic acid	29.1	799	236, 274	647 , 495
27	Trigalloyl-mono(digalloyl) quinic acid	29.2	951	236, 274	799, 647, 495
28	Tetragalloyl quinic acid	29.6	799	236, 274	647 , 495
29	Trigalloyl quinic acid	29.8	647	238, 276	495 , 343, 169
30	Tetragalloyl quinic acid	30.0	799	236, 274	647 , 495
31	Tetragalloyl quinic acid	30.1	799	236, 274	647 , 495
32	Trigalloyl quinic acid	30.1	647	238, 276	495 , 343, 169
34	Tetragalloyl quinic acid	30.7	799	236, 274	647 , 495
36	Trigalloyl-mono(digalloyl) quinic acid	31.2	951	236, 274	799, 647, 495
37	Trigalloyl-mono(digalloyl) quinic acid	31.5	951	236, 274	799, 647, 495
38	Trigalloyl-mono(digalloyl) quinic acid	32.1	951	236, 274	799, 647, 495
39	Trigalloyl-mono(digalloyl) quinic acid	32.5	951	236, 274	799, 647, 495
40	Trigalloyl-mono(digalloyl) quinic acid	32.7	951	236, 274	799, 647, 495
41	Trigalloyl-mono(digalloyl) quinic acid	33.0	951	236, 274	799, 647, 495
42	Digalloyl-di(digalloyl) quinic acid	33.4	1103	238, 274	951, 799 , 647
43	Digalloyl-di(digalloyl) quinic acid	33.7	1103	238, 274	951, 799 , 647
44	Digalloyl-di(digalloyl) quinic acid	33.9	1103	238, 274	951, 799 , 647
45	Digalloyl-di(digalloyl) quinic acid	34.1	1103	238, 274	951, 799 , 647
46	Digalloyl-di(digalloyl) quinic acid	35.0	1103	238, 274	951, 799 , 647
47	Digalloyl-di(digalloyl) quinic acid	35.1	1103	238, 274	951, 799 , 647
48	Digalloyl-di(digalloyl) quinic acid	35.5	1103	238, 274	951, 799 , 647
49	Digalloyl-di(digalloyl) quinic acid	35.7	1103	238, 274	951, 799 , 647
50	Digalloyl-di(digalloyl) quinic acid	36.1	1103	238, 274	951, 799 , 647
51	Galloyl-tri(digalloyl) quinic acid	37.1	1255	256, 279	1103 , 951, 799, 647
52	Galloyl-tri(digalloyl) quinic acid	37.4	1255	256, 279	1103 , 951, 799, 647
<i>Gallic acid derivatives</i>					
1	Gallic acid	9.1	169	272	169, 125
<i>Proanthocyanidins</i>					
3	Gallocatechin	13.7	305	268	261, 219, 179 , 125
8	Gallocatechin-dimer	18.2	611	240, 272	547, 305 , 219
10	Catechin-dimer	20.7	577	238, 274	559, 451 , 425, 289, 245
11	Catechin-dimer	21.6	577	238, 274	559, 451 , 425, 289, 245
14	Gallocatechin-gallate-dimer	23.9	915	240, 274	457 , 305
17	Gallocatechin-gallate	25.8	457	238, 276	331, 305, 169
35	Catechin-gallate-dimer	30.8	883	238, 278	441 , 289

571 Table 3. Low molecular weight phenols detected by HPLC-DAD-ESI-MS-MS in the antioxidant
572 formulas.

Number	Compound	AO1	AO2 (mg/100g powder)	AO3
<i>Flavonols</i>				
53	Quercetin 3- <i>O</i> -glucoside	n.d.	n.d.	12.55 ± 0.96
54	Kaempferol 3- <i>O</i> -hexosyl-rhamnosil-hexose	n.d.	n.d.	2.70 ± 0.20
55	Myricetin	n.d.	n.d.	16.77 ± 0.40
56	Quercetin	29.09 ± 0.64	9.77 ± 0.75	98.81 ± 0.18
57	Kaempferol	10.91 ± 0.92	0.22 ± 0.12	49.47 ± 1.06
Total		40.00 ± 1.56	9.99 ± 0.89	180.30 ± 2.92
<i>Ellagitannins and ellagic acid</i>				
2	Vescalagin	689.45 ± 2.87	n.d.	n.d.
4	Castalagin	636.23 ± 4.1	n.d.	n.d.
5	Hexahydroxy-diphenoyl-galloyl-glucose	42.72 ± 1.52	n.d.	n.d.
6	Hexahydroxy-diphenoyl-galloyl-glucose	40.44 ± 1.28	n.d.	n.d.
33	Ellagic acid	95.22 ± 1.91	n.d.	n.d.
Total		1504.06 ± 11.93	--	--
<i>Gallotannins</i>				
7	Digalloyl quinic acid	283.73 ± 17.76	n.d.	n.d.
9	Digalloyl quinic dimer	827.95 ± 10.28	176.34 ± 8.51	n.d.
12	Trigalloyl quinic acid	773.02 ± 17.16	134.71 ± 1.45	n.d.
13	Trigalloyl quinic acid	1893.72 ± 68.63	326.79 ± 2.71	n.d.
15	Trigalloyl quinic acid	1032.30 ± 13.85	167.58 ± 1.78	n.d.
16	Trigalloyl quinic acid	n.d.	67.18 ± 2.31	n.d.
18	Digalloyl quinic acid	456.95 ± 1.84	78.88 ± 1.60	n.d.
19	Tetragalloyl quinic acid	382.10 ± 18.13	n.d.	n.d.
20	Tetragalloyl quinic acid	n.d.	52.72 ± 0.83	n.d.
21	Trigalloyl quinic acid	180.00 ± 8.71	n.d.	n.d.
22	Tetragalloyl quinic acid	618.35 ± 5.82	n.d.	n.d.
23	Tetragalloyl quinic acid	n.d.	92.16 ± 2.41	n.d.
24	Trigalloyl quinic acid	797.50 ± 21.63	n.d.	n.d.
25	Tetragalloyl quinic acid	548.06 ± 10.70	n.d.	n.d.
26	Tetragalloyl quinic acid	n.d.	173.94 ± 1.84	n.d.
27	Trigalloyl-mono(digalloyl) quinic acid	472.76 ± 3.50	n.d.	n.d.
28	Tetragalloyl quinic acid	n.d.	20.14 ± 0.73	n.d.
29	Trigalloyl quinic acid	562.67 ± 20.21	n.d.	n.d.
30	Tetragalloyl quinic acid	269.70 ± 7.31	n.d.	n.d.
31	Tetragalloyl quinic acid	n.d.	26.30 ± 0.80	n.d.
32	Trigalloyl quinic acid	139.49 ± 4.72	n.d.	n.d.
37	Trigalloyl-mono(digalloyl) quinic acid	247.36 ± 12.66	26.96 ± 2.05	n.d.
38	Trigalloyl-mono(digalloyl) quinic acid	246.99 ± 3.30	23.02 ± 0.47	n.d.
39	Trigalloyl-mono(digalloyl) quinic acid	521.57 ± 5.5	59.33 ± 2.15	n.d.
40	Trigalloyl-mono(digalloyl) quinic acid	129.19 ± 9.77	n.d.	n.d.
41	Trigalloyl-mono(digalloyl) quinic acid	26.13 ± 1.41	n.d.	n.d.
42	Digalloyl-di(digalloyl) quinic acid	62.52 ± 1.48	n.d.	n.d.
43	Digalloyl-di(digalloyl) quinic acid	54.74 ± 1.24	n.d.	n.d.
44	Digalloyl-di(digalloyl) quinic acid	45.87 ± 0.84	n.d.	n.d.
45	Digalloyl-di(digalloyl) quinic acid	42.02 ± 2.89	n.d.	n.d.
46	Digalloyl-di(digalloyl) quinic acid	114.07 ± 6.48	n.d.	n.d.
47	Digalloyl-di(digalloyl) quinic acid	61.43 ± 5.39	n.d.	n.d.
48	Digalloyl-di(digalloyl) quinic acid	52.93 ± 2.53	n.d.	n.d.
49	Digalloyl-di(digalloyl) quinic acid	34.01 ± 4.11	n.d.	n.d.
50	Digalloyl-di(digalloyl) quinic acid	20.73 ± 2.8	n.d.	n.d.
51	Galloyl-tri(digalloyl) quinic acid	72.71 ± 3.53	n.d.	n.d.
52	Galloyl-tri(digalloyl) quinic acid	35.61 ± 1.17	n.d.	n.d.
Total		11006.18 ± 184.09	1426.05 ± 14.18	--
<i>Gallic acid derivatives</i>				
1	Gallic acid	1147.05 ± 14.87	727.47 ± 2.68	2121.62 ± 21.08
<i>Proanthocyanidins</i>				
3	Gallocatechin	n.d.	n.d.	49.53 ± 5.4
8	Gallocatechin-dimer	n.d.	55.88 ± 2.03	190.34 ± 1.40

10	Catechin-dimer	128.29 ± 3.43	155.87 ± 2.30	582.06 ± 10.11
11	Catechin-dimer	n.d.	n.d.	121.68 ± 5.92
14	Gallocatechin-gallate-dimer	1255.60 ± 72.97	1714.41 ± 7.96	4570.72 ± 77.15
17	Gallocatechin-gallate	n.d.	n.d.	328.62 ± 4.95
35	Catechin-gallate-dimer	721.53 ± 17.24	499.97 ± 1.56	1918.53 ± 61.34
Total		2105.42 ± 52.3	2426.13 ± 9.25	7761.48 ± 20.08
Total phenols		15802.51 ± 378.73	4589.64 ± 30.17	10063.4 ± 23.12

573 Table 4. Content of low molecular weight phenols in the antioxidant formulas. Data are reported as
574 mean values (n=2) ±standard deviation; n.d.: not detected.

575

576

Formula	Sotolon	Glutathione	Cysteine		Ascorbic acid	Dehydroascorbic acid	Antioxidant capacity		DPPH/TP ratio	ABTS/TP ratio
			Free	Absorbed			DPPH	ABTS		
			mg /100 g powder							
AO1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8776±650	1660±109	151.8	28.7
AO2	n.d.	n.d.	n.d.	9.0±0.28	n.d.	n.d.	5990±443	1338±98	117.7	26.3
AO3	n.d.	5.77±0.18	n.d.	64.5±2.0	n.d.	n.d.	1768±131	133±10	124.5	9.4

577 Table 5: Content of sotolon, glutathione, free and adsorbed cysteine, ascorbic acid, dehydroascorbic
578 acid and antioxidant capacity of the antioxidant formulas. Data are reported as mean values (n=3)
579 ±standard deviation; n.d.: not detected.

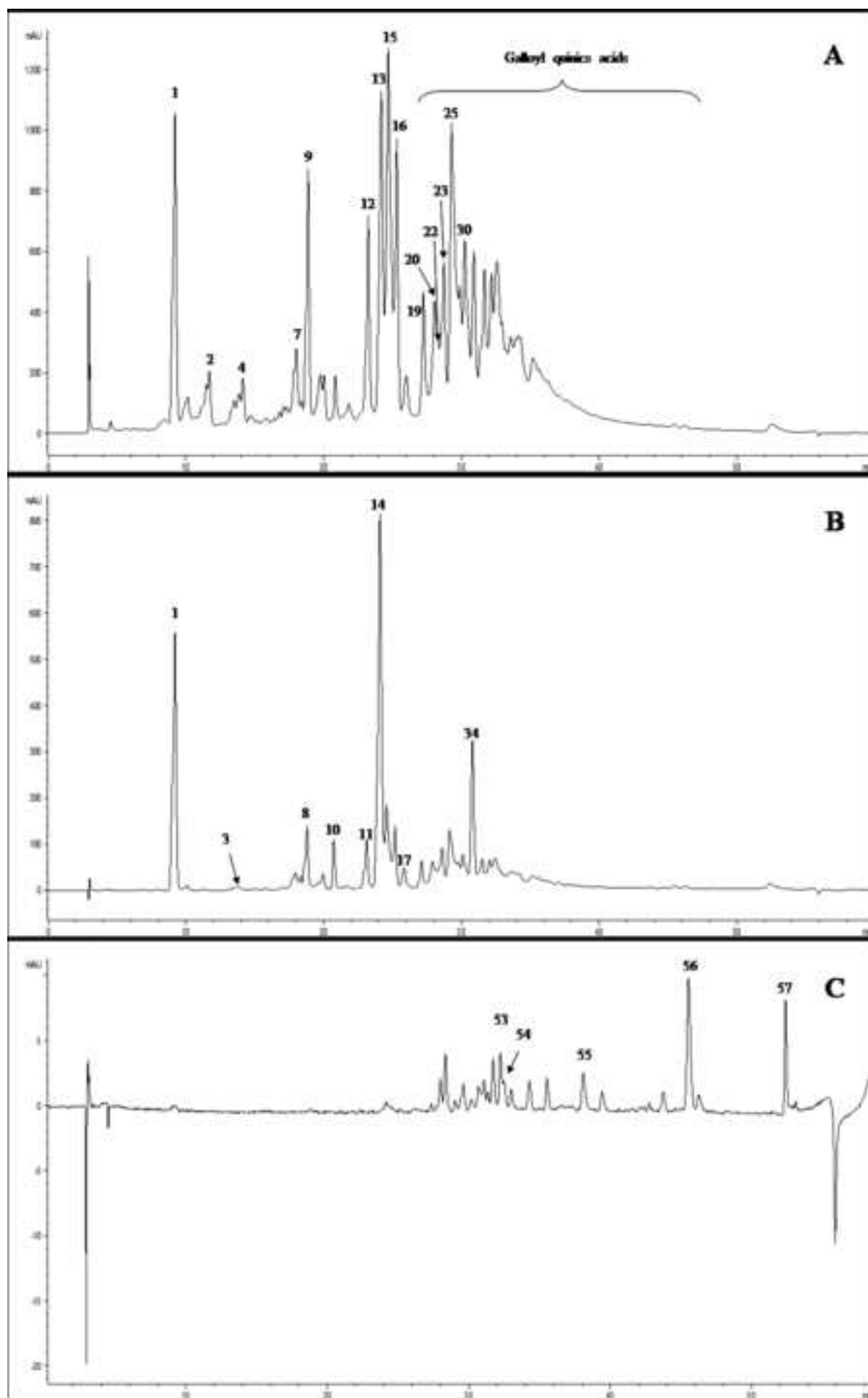
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Antioxidant added	Dosage (mg/l)	Storage temperature (°C)	Total phenols (mg gallic acid/l)	Absorbance 420 nm	Glutathione (mg/l)	2-S-glutathionyl caftaric acid (mg/l)	Sotolon (µg/l)
No addition	--	15	118.5±9.5 ^a	0.142±0.001 ^a	1.33±0.040 ^a	0.57±0.018 ^a	n.d.
No addition	--	25	124.5±10.0 ^a	0.150±0.001 ^a	2.66±0.082 ^a	0.41±0.013 ^a	n.d.
SO ₂	50	15	119.7±9.6 ^a	0.099±0.001 ^a	0.93±0.029 ^a	0.50±0.016 ^a	n.d.
SO ₂	50	25	122.8±9.8 ^a	0.122±0.002 ^a	2.25±0.068 ^a	0.47±0.015 ^a	n.d.
AO1	20	15	131.4±10.5 ^b	0.154±0.001 ^b	1.80±0.054 ^a	0.54±0.017 ^a	n.d.
AO1	20	25	136.1±10.8 ^b	0.158±0.000 ^b	3.58±0.11 ^a	0.41±0.013 ^a	< 2
AO1	40	15	143.1±11.4 ^b	0.156±0.000 ^b	2.13±0.066 ^a	0.70±0.021 ^a	n.d.
AO1	40	25	147.4±11.8 ^b	0.160±0.000 ^b	3.38±0.10 ^a	0.61±0.020 ^a	< 2
AO2	20	15	131.4±10.5 ^b	0.157±0.000 ^b	1.31±0.041 ^a	0.72±0.022 ^a	< 2
AO2	20	25	132.5±10.6 ^b	0.172±0.000 ^b	2.37±0.073 ^a	0.53±0.017 ^a	< 2
AO2	40	15	133.9±10.6 ^b	0.170±0.001 ^b	1.39±0.043 ^a	0.57±0.017 ^a	6.41±0.11
AO2	40	25	145.8±11.2 ^b	0.181±0.002 ^b	4.26±0.076 ^a	0.35±0.011 ^a	13.37±0.22
AO3	20	15	119.2±9.5 ^a	0.147±0.001 ^a	1.57±0.049 ^a	0.56±0.016 ^a	n.d.
AO3	20	25	121.2±9.7 ^a	0.147±0.000 ^a	2.51±0.079 ^a	0.45±0.014 ^a	< 2
AO3	40	15	127.2±9.9 ^a	0.141±0.000 ^a	1.58±0.049 ^a	0.58±0.017 ^a	n.d.
AO3	40	25	125.1±10.0 ^a	0.155±0.000 ^a	3.78±0.12 ^a	0.45±0.014 ^a	< 2

Table 6: Concentration of glutathione, 2-S-glutathionyl caftaric acid and sotolon, and absorbance values in sparkling white wine samples stored under different conditions. Data are reported as mean values (n=3) ±standard deviation; n.d.: not detected. Different letters mean significant difference ($p < 0.05$).

Figure

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Highlights

- Addition of polyphenols formulas was investigated in sparkling white wine after disgorging.
- Certain markers of oxidation were evaluated in comparison to sulfur dioxide.
- The formulas seemed less protective against the oxidation than sulfur dioxide.
- The knowledge of the phenolics in the formulas allows their proper use in winemaking.